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Review

Autonomic drugs in the treatment of canine and feline glaucoma – Part II: Medications that lower intraocular pressure by reducing aqueous humour production

T. Maślanka

Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Warmia and Mazury, Oczapowskiego 13, 10-718 Olsztyn, Poland

Abstract

One characteristic of the most common types of glaucoma is increased intraocular pressure (IOP), which has a damaging effect on optic nerve axons, leading to progressive loss of retinal ganglion cells. Therefore, ocular hypotensive drugs are the mainstay of pharmacological therapy for glaucoma. This review article, which is the second part of a two-part series, is dedicated to autonomic drugs which lower IOP by decreasing the aqueous humour production. These agents are subdivided into two groups: β-adrenergic antagonists and selective α_2-adrenergic agonists. This paper summarizes the current state of knowledge on the mechanism of action of these drugs and their effect on IOP in dogs and cats. Moreover, it discusses their possible undesirable side effects of these medications and presents the current ideas about their role and position in the medical management of glaucoma in small animals.

Key words: glaucoma, IOP, β-adrenergic antagonists, selective α_2-adrenergic agonists, dogs, cats

β-adrenergic antagonists (also known as β-blockers, β-antagonists, or β-adrenergic blocking agents)

β-blockers are competitive antagonists of β-adrenergic receptors. They can be divided into two groups based on their selectivity for β-adrenergic receptors:

– Nonselective β-adrenergic antagonists: timolol, levobunolol, metipranolol and carteolol; carteolol is the only topical β-blocker which possesses intrinsic sympathomimetic activity (ISA); they bind equally well to both β_1- and β_2-adrenergic receptors.

– Selective β_1-adrenergic antagonists: betaxolol; however, the selectivity for β_1-adrenergic receptors is not absolute (some inhibitory effects on β_2 receptors can be expected).

β-antagonists were first reported to lower intraocular pressure (IOP) in humans in 1967 when Phillips et al. (1967) described the ocular hypotensive effect of intravenously administered propranolol. One year later, topical propranolol was also reported to

Correspondence to: T. Maślanka, e-mail: tomasz.maslanka@uwm.edu.pl, tel.: +48 89 523 37 58

reduce IOP (Bucci et al. 1968). Unfortunately, potential development of propranolol as a topical ocular hypotensive agent was limited by unwanted corneal property (Musini et al. 1971). Other β-blockers known at that time also demonstrated unwanted side effects, which prohibited safe use of these drugs as topical antiglaucoma agents. The breakthrough occurred in 1976-1977 upon the discovery that timolol markedly reduced IOP in both healthy humans (Katz et al. 1976) and in glaucoma patients (Zimmerman and Kaufman 1977). In 1978, timolol maleate was introduced as the first topical ocular hypotensive β-adrenergic receptor blocker. Timolol revolutionized glaucoma management because it demonstrated better efficacy and minimal ocular adverse effects compared to other anti-glaucoma agents available those days (Boger et al. 1978a,b, Sonntag et al. 1979). Thereafter, in 1980s and early 90s several topical β-blockers were introduced into the market. Since the introduction of these agents for ophthalmic use in 1979, they were the routine choice as first-line agents for IOP lowering in primary open-angle glaucoma (POAG) in humans. When prostaglandin $F_2\alpha$ analogues (PGAs) became commercially available, the role of β-blockers in therapy of human glaucoma substantially diminished, although they remain important and are still commonly administered as ocular hypotensive agents.

Mechanism of action

It is widely accepted that β-blockers lower IOP through reduction of aqueous humour (AH) formation, but the exact mechanism responsible for inducing this effect has not been determined in an univocal and unambiguous manner. Back in the 1970s and 1980s, fluorophotometric studies in humans showed that topical β-blockers reduced AH secretion by 24% to 47% (Coakes and Brubaker 1978, Yablonski et al. 1978, Dailey et al. 1982, Reiss and Brubaker 1983). In addition, results of studies conducted on cats (Helal et al. 1979, Liu et al. 1980), monkeys (Takase 1976) and rabbits (Liu et al. 1984) show that the IOP-lowering effect of β-blockers is the consequence of depressed AH production and is unrelated to enhancement of AH outflow facility. It is noteworthy that except very few cases the vast majority of studies demonstrate that these drugs do not affect or else produce just a minimal effect on AH outflow facility (Bonomi and Steindler 1975, Tieri and Polzella 1975, Zimmerman et al. 1977).

The widely accepted concept is that by blocking β_2-receptors the drugs discussed abolish the adrenergic mediated stimulation of AH production, which consequently leads to the inhibition of this process. The following evidence supports the claim:

– There are many findings indicating that AH formation is under adrenergic control (Reiss et al. 1984, Topper and Brubaker 1985, McCannel et al. 1992).

– β-adrenergic receptors are present in the ciliary processes (which are the site of AH production) in humans (Wax and Molinoff 1987), rabbits (Bromberg et al. 1980) and cattle (Elena et al. 1984), and they are predominantly β_2-subtype (Nathanson 1980, Elena et al. 1984, Wax and Molinoff 1987, Crook and Riese 1996).

– β-adrenergic antagonists suppress (Takase 1976, Coakes and Brubaker 1978, Yablonski et al. 1978, Helal et al. 1979, Liu et al. 1980, Dailey et al. 1982, Reiss and Brubaker 1983, Liu et al. 1984), while the agonists [salbutamol (Miichi and Nagataki 1983, Coakes and Siah 1984) and terbutaline (Gharagozloo et al. 1988a)] enhance AH formation.

– β-adrenergic antagonists inhibit the actions of β-adrenergic agonists on AH dynamics (Takase 1976, Bartels et al. 1980).

– β-adrenergic antagonists either do not affect or produce a very weak effect on the ciliary epithelium enzymes and synthetic pathways connected with AH formation, i.e. Na^+/K^+-ATPase, Mg^{2+}-ATPase, and prostaglandin biosynthesis (Watanabe and Chiou 1983); what is more, it has been recently demonstrated that timolol activates the enzyme activities of human CA-II (Sugimoto et al. 2010).

It is uncertain whether the IOP-lowering effect of β-blockers is a result of the inhibition of the classical β-adrenergic signalling pathway [i.e. *via* adenylate cyclase and cyclic adenosine monophosphate (cAMP)], because it is unclear if the cAMP signalling pathway mediates regulation of AH production at all. It has been shown that epinephrine raises the cAMP level in AH (Boas et al. 1981). This finding is congruent with results of other studies, in which it has been found that the incubation of excised ciliary processes or cultured ciliary epithelial cells with terbutaline (selective β_2-adrenergic receptor agonist) produced concentration-dependent increases in cyclic AMP in both tissues (Shahidullah et al. 1995). Those results would suggest that the blockade of β-adrenergic receptors should lead to reduction of the production of cAMP, but it has been demonstrated that timolol treatment does not affect the level of cAMP in AH. In addition, there are results indicating that direct stimulation of cAMP production leads to inhibition of AH formation; it was demonstrated that forskolin considerably reduced AH production, which was correlated with adenylate cyclase activation and increased cAMP concentration in AH (Caprioli et al. 1984, Bartels et al. 1987). From

the above results, it can be concluded that alterations in total cellular cAMP concentration do not mediate the drug-triggered changes in AH formation (Toris 2010). Two explanations have been proposed to explain these inconsistencies (Macknight and Civan 2008). First, β-blocker-induced reduction in AH production might be mediated by reducing cAMP, but that action is exerted at a compartmentalized membrane site, so that flooding the entire cell with cAMP triggers many additional unrelated and confounding effects (Macknight and Civan 2008). Another possibility is that β-blocker's-action on AH formation may be mediated by blockade of β-adrenergic receptors, but through the arachidonic acid signaling cascade by coupling to G_i proteins (Civan 2008). It is worth considering as an option that the blockade of classical β-adrenergic receptors may not be involved in the β-blocker-induced reduction in AH production, but that some other mechanisms mediate the effect discussed. Macknight and Civan (2008) have suggested that inhibition of Cl^-/HCO_3^- exchange may mediate timolol's inhibition of AH formation. It is possible that β-blockers reduce AH production through both the blockade of classical β-adrenergic receptors and *via* other mechanisms, such as the aforementioned inhibition of Cl^-/HCO_3^- exchange.

Effect on IOP

The available literature contains several reports discussing the influence of timolol on IOP in normotensive and glaucomatous dogs and cats (Gum et al. 1991, Wilkie and Latimer, 1991a,b, Gelatt et al. 1995, Maehara et al. 2004, Plummer et al. 2006, Dietrich et al. 2007, Smith et al. 2010) and one report on the efficacy of betaxolol in prophylactic antiglaucoma therapy in primary angle closure glaucoma (PACG) in dogs (Miller et al. 2000). To the best of the author's knowledge, there are no published data on the influence of other topical β-blockers on IOP in small animals. An experiment by Wilkie and Latimer (1991a) on the effect of locally applied timolol maleate (0.5%) on IOP in normotensive dogs showed that administration of a single dose of the drug considerably reduced IOP in the treated and untreated eyes. The mean reduction of IOP was 16.1% in the treated eye and 9.0% in the nontreated eye; the maximum IOP reduction effect occurred within 2 to 4 h of instillation. The authors concluded that these results support the use of timolol for treatment of glaucoma in dogs (Wilkie and Latimer 1991a). Maehara et al. (2004) also administered timolol for 28 days in clinically normal dogs and demonstrated that the medicine significantly reduced IOP: pre-administration IOP was 15.6 mmmHg and on the 28th day was 11.5 mmHg. The maximum per cent decrease in IOP was observed on the 7th day and was 27.1% (Maehara et al. 2004). Nevertheless, the cited results on the effect of timolol on IOP in normotensive dogs disagree with results obtained by other researchers (Gum et al. 1991, Gelatt et al. 1995, Smith et al. 2010). Smith et al. (2010) did not demonstrate any larger IOP reduction in clinically normal dogs following the administration of timolol (0.5%). Likewise, another team of scientists did not observe timolol (0.25% and 0.5%) causing an IOP-lowering effect in healthy Beagles (Gum et al. 1991). Even topical administration of timolol in such high concentrations as 4% and 6% did not cause consistent reductions in IOP in clinically normal dogs (Gelatt et al. 1995). In the Beagles with glaucoma, concentrations of timolol 4% and 6% produced a decrease in IOP ranging from 8 to 14 mmHg in the treated eye (Gelatt et al. 1995). However, it must be added that due to the inhibitory effect of timolol on the heart rate, administration of such high doses of the preparation is not safe. The quoted studies have demonstrated that administration of timolol (in all the tested doses) coincided with a considerable decrease in the heart rate. Timolol at commercially available concentrations (0.25% and 0.5%) also reduces IOP in glaucomatous dogs, although the effect is not as profound as that induced by the application of the drug at higher concentrations such as 4%, 6% and 8%. It was determined that timolol reduced IOP by approximately 4 to 5 mmHg in Beagles with open angle glaucoma (Gum et al. 1991). This is supported by results of other investigators (Plummer et al. 2006), who conducted comparative studies on the effect of the topical administration of a fixed combination of dorzolamide (carbonic anhydrase inhibitor) and timolol and monotherapy with timolol or dorzolamide on IOP in Beagles with inherited POAG. They found that timolol had a IOP-lowering effect, although corresponding to just half of the effect achieved by administration of dorzolamide. Timolol alone, dorzolamide alone, and the combination of the two decreased IOP after 1 day of treatment 2.83 mmHg, 6.47 mmHg, and 6.56 mmHg, respectively. After 4 days of treatment, the IOP decreased even further: timolol alone, dorzolamide alone, and the combination of the two decreased IOP 3.75 mmHg, 7.50 mmHg, and 8.42 mmHg, respectively. These results confirm the broadly accepted notion that in dogs dorzolamide has a stronger effect on the reduction of IOP than timolol. Furthermore, they prove that combined administration of dorzolamide and timolol in dogs has additive effects. Although both agents are suppressors of AH formation, the occur-

rence of the additive ocular hypotensive effect between them is expected, because they inhibit AH production through different target sites. Timolol has also been shown to have the additive effect with pilocarpine in glaucomatous Beagles (Gelatt et al. 1995). The ocular hipotensive effect of β-blockers in man is generally additive to that of carbonic anhydrase inhibitors (CAIs) (Wayman et al. 1997), parasympathomimetics (Zadok et al. 1994) and PGAs (Higginbotham et al. 2002). In the human medicine it is thought that β-blockers can be used with virtually any other ocular hypotensive agents, with the expectation that some additional pressure-lowering effect will be obtained. The only exception is the non-selective adrenergic agonists such as epinephrine and dipivefrine, for which the additive effect to the non-selective β-adrenergic antagonists is minimal (Stamper et al. 2009). Considering the existing differences between man and small animals in respect of the response to particular groups of ocular hipotensive drugs, caution should be taken when trying to extrapolate from data on humans to small animals. Recapitulating, results of studies on the effect of timolol on IOP in glaucomatous dogs are relatively congruent in that they all indicate that the drug lowers IOP in dogs suffering from glaucoma but the effect is relatively weak.

Wilkie and Latimer (1991b), who studied the effect produced by locally administered timolol maleate (0.5%) on IOP in normotensive cats, showed that the administration of a single dose largely reduced IOP in the treated and untreated eyes. In cats, the mean reduction of IOP was 22.3% in the treated eye and 16.3% in the nontreated eye; the maximum IOP reduction effect occurred within 6 to 12 h of instillation. The authors concluded that these results support the use of timolol for treatment of glaucoma in cats (Wilkie and Latimer 1991b). No significant additive ocular effect was detected in normotensive cats treated with a combination of dorzolamide (2%) and timolol (0.5%) relative to the IOP-lowering effect of treatment with dorzolamide alone (Dietrich et al. 2007). These data indicate that a CAI alone can maximally suppress AH formation in cats (McLellan and Miller 2011). As β_2-adrenergic receptors predominate in the feline anterior segment (Colasanti and Trotter 1981), β_1-selective blockers, i.e. betaxolol, may have lower efficacy in this species (McLellan and Miller 2011).

As β-blockers do not reduce AH formation during sleep (because the sympathetic activity is minimal during sleep) (Liu et al. 1984, Topper and Brubaker 1985), their reduced effectiveness during the night-time should also be taken into account in the animals which sleep much during the daytime.

Clinical use

In people, β-blockers have their greatest use in the treatment of POAG. They are also effective in reducing IOP in most of the secondary glaucomas. Moreover, β-blockers can be used as adjunctive therapy in angle-closure glaucoma (Barlett et al. 2008, Stamper et al. 2009). Their importance in the medical management of glaucoma in small animals, especially dogs, is much smaller, also because the reduction of IOP in these patients by β-blockers is relatively poor.

Gelatt et al. (2007) frequently use the commercially available 0.5% timolol maleate q 12 h in canine glaucoma patients. Another researcher (Willis 2004) also suggests that 0.25% and 0.5% timolol, as well as 0.25% and 0.5% betaxolol might be helpful in treatment of acute and chronic (primary and secondary) glaucoma in small animals. However, Petersen-Jones and Stanley (2009) underline that β-blockers may be a useful adjunct to therapy, but their effect is insufficient to warrant their sole use. Since these drugs do not exacerbate intraocular inflammation, they can be suitable for treatment of glaucoma in cats.

Certainly, β-blockers are not the primary drugs for emergency therapy in patients with acute glaucoma, but should be treated exclusively as adjunctive medications, applied to enhance the reduction of IOP induced by other ocular hypotensive drugs (mainly CAIs and PGAs). According to the treatment algorithms for various types of canine and feline glaucoma recommended in Slatter's Fundamentals of Veterinary Ophthalmology (Miller 2008), 0.5% timolol (every 8 to 12 h) is the second-line drug for emergency therapy of uveitis-induced glaucoma and hyphema-associated glaucoma, if the expected IOP reduction has not been achieved *via* administration of a CAI drug. Alternatively, in these cases the application of the combination of 2% dorzolamide with 0.5% timolol can be used as the first-line treatment option (Miller 2008). The algorithm for the management of patients with acute PACG presented in the aforementioned book does not include application of β-blockers to the affected eye, although it recommends prophylactic therapy of the fellow normotensive eye consisting of the application of 0.5% betaxolol every 12 h. These agents may have a role in prophylactic antiglaucoma therapy of PACG in dogs. Miller et al. (2000) evaluated the ability of 0.5% betaxolol to prevent glaucoma in the fellow eye of dogs with unilateral PACG in a multicenter, open-label, clinical trial. They found that treatment with betaxolol prolonged the time to onset of PACG (median: 30.7 months), compared with untreated dogs (median: 8 months). These results suggest that be-

taxolol can be an efficient drug to prevent or delay the onset of clinical disease in an eye predisposed to PACG.

Side effects

In the human medicine topical β-blockers are well tolerated by most patients (Marquis and Whitson 2005). Reported local side effects include stinging, conjunctival hyperemia, superficial punctate keratitis, photophobia and worsening dry eye symptoms (Coakes et al. 1981). During the administration of timolol in dogs and cats, distinct reduction in the pupil size occurs although the same drug given to people has a negligible effect on the pupillary diameter (Wilkie and Latimer 1991a,b, Gelatt et al. 1995, Plummer et al. 2006, Smith et al. 2010). Apart from the impact on the pupil size, we lack data concerning local adverse effects attributed to the use of β-blockers in small animals.

β-blockers may cause systemic side-effects in glaucoma patients due to absorption of the drug into systemic circulation. They can exert serious systemic adverse effects from blockade of β_1-adrenergic receptors of the heart and β_2-adrenergic receptors of the respiratory smooth muscle. Bradycardia, cardiac block, lower blood pressure, congestive heart failure and bronchospasm have been reported following topical nonselective β-blockers in humans (McMahon et al. 1979, Juzych et al. 1997). Therefore, in people topical β-blockers are contraindicated in patients with asthma, severe chronic obstructive pulmonary disease, bradycardia, severe heart block and overt cardiac failure (Bartlett et al. 2008). It should be assumed that the above undesirable side effects can also appear in small animals. It has been found that topical application of 0.5% timolol in dogs is associated with a significant reduction in heart rate (Maehara et al. 2004, Plummer et al. 2006, Smith et al. 2010) and blood pressure (Maehara et al. 2004).

Selective α_2-adrenergic agonists

The prototype α_2-agonist is clonidine, a centrally acting adrenergic agonist introduced into clinical practice in 1966 as an anti-hypertensive agent. In the same year, it was demonstrated that i.v. administration of the drug significantly reduced IOP (Makabe 1966). Three years later the first report was published stating that also topical administration of clonidine to the eye induced the same effect (Hasslinger 1969), the fact which was later verified by other researchers (Harrison and Kaufmann 1977). Clonidine was used as an ocular hypotensive agent in many countries but due to serious adverse systemic side effects (i.e. systemic hypotension, bradycardia and sedation) its application in the therapy of glaucoma has been abandoned. The above side effects appear because this drug easily penetrates the blood-brain barrier (BBB), which is associated with its lipophilic character. Presently, the drugs belonging to selective α_2-adrenergic agonists that are available and prescribed in treatment of glaucoma are apraclonidine and brimonidine. Apraclonidine is hydrophilic derivative of clonidine and produces substantial IOP reduction without causing the centrally mediated side effects typical for its parent drug. Apraclonidine differs from clonidine by the addition of an amide group at the C4 position of the benzene ring. This modification makes the drug less lipophilic and therefore less able to have significant BBB penetration, which reduces the risk of systemic side effects (Reynolds 2009). Brimonidine is less lipophilic than clonidine but more than apraclonidine. Penetration of BBB is probably intermediate compared to the other two drugs and centrally mediated side effects are probably more of an issue partly because of this (Reynolds 2009).

Although clonidine, apraclonidine and brimonidine are commonly classified as selective α_2-adrenergic agonists, these drugs demonstrate some α_1-activity. In fact, apraclonidine is moderate α_2-selective agent, because its affinity to α_2 receptors is just 72-fold higher that the affinity to α_1 receptors (Coleman et al. 1989, Reynolds 2009). Conversely, brimonidine is a highly selective α_2-agonist that is 1000-fold more selective for α_2- versus α_1-receptors, and is 7-12-fold more α_2-selective than clonidine and 23- to 32-fold more α_2-selective than apraclonidine (Burke and Schwartz 1996). This is the reason why many authors classify clonidine and apraclonidine as relatively selective α_2-adrenergic agonists.

Mechanism of action

Results of numerous studies indicate that α_2-agonists lower IOP mostly by decreasing AH production. Lee et al. (1984), having tested the effect of clonidine on the rate of AH flow in the eyes of normal human subjects, concluded that the value of this parameter was 21% lower in clonidine-treated eyes as compared to fellow placebo-treated eyes (it is generally assumed that changes in aqueous flow reflect changes in AH production). Apraclonidine and brimonidine have also been demonstrated to decrease aqueous flow, although the scale of this effect differs significantly when results reported by different research teams are compared. Toris et al. (1995b) demonstrated that

topical instillation of apraclonidine (0.5%) reduced AH flow by 12%. Gharagozloo et al. (1988b) found that 4 h after topical administration of apraclonidine (1%) to one eye of normal human subjects, the flow rate in the apraclonidine-treated eyes was 35% lower than that measured in the control eyes. This finding in congruent with results of later studies, when it was determined that topical instillation of apraclonidine (0.5%) suppressed AH flow between 39% and 44% (Schadlu et al. 1998). In the same studies, it has been shown that brimonidine (0.2%) reduced AH flow between 44% and 48%. In turn, many researchers found out that topical administration of brimonidine (0.2%) to eyes of healthy human subjects reduced the aqueous humour flow by 20% (Toris et al. 1995a), 33% (Larsson 2001) and 28% (Tsukamoto and Larsson 2004). No mechanism has been determined that mediates reduction of AH formation under the influence of α_2-agonists, although it should be assumed that by virtue of its nature the effect is achieved *via* α_2-adrenergic receptor stimulation. α_2-adrenergic receptors are located presynaptically and postsynaptically. In general, presynaptic α_2-adrenergic receptor subtypes act as inhibitory autoreceptors to control neurotransmitter (e.g. norepinephrine) release, while postsynaptic α_2-adrenergic receptor stimulation causes a similar action with α_1-stimulation. It needs to be mentioned that both the function and the biochemical nature of postsynaptic α_2-adrenergic receptors are poorly recognized, especially with respect to the eye. This is one of the reasons why at the current state of knowledge it is impossible to present in greater detail the mechanism responsible for the ocular hypotensive effect of α_2-agonists. α_2-adrenergic receptors have been identified on presynaptic adrenergic nerve terminals and postsynaptically in the ciliary body (Crosson et al. 1992, Huang et al. 1995, Bartlett et al. 2008). Theoretically, considering the localization of α_2-adrenergic receptors (against the synapsis), α_2-agonists can inhibit AH production through:

– Activation of the presynaptic α_2-receptors, which inhibits release of norepinephrine: the amount of norepinephrine available for other adrenergic receptors, including β-receptors on the ciliary epithelium, is decreased (Bartlett et al. 2008). Thus, a reduction of the sympathetic tone at the level of ciliary body takes place, which may lead to the inhibition of AH formation, because – as mentioned before – there are many findings implying that this process is under adrenergic control (Reiss et al. 1984, Topper and Brubaker 1985, McCannel et al. 1992). Such an explanation of the mechanism of action is accepted (Bartlett et al. 2008), despite lacking strong, direct proofs to confirm it. Nonetheless, data reported by many researchers (Allen and Langham 1976, Burke and Potter 1986, Jumblatt et al. 1987) support this sequence of events. The results obtained by Jumblatt et al. (1987) indicate that the rabbit iris-ciliary body contains functional, presynaptic α_2-receptors which may play an autoregulatory role *in vivo* and contribute to the ocular effects of adrenergic drugs. In another study on rabbits it was demonstrated that the hypotensive response to clonidine was dependent on intact adrenergic innervation of the ocular tissues (Allen and Langham 1976). In turn, investigations carried out on cats, rabbits and monkeys showed that a relatively selective α_2-agonist UK-14, 304-18, lowers IOP, in part, by suppressing sympathetic neuronal function (Burke and Potter 1986). However, the results attained by Gabelt et al. (1994) undermine the above, proving that reduction in AH flow produced by both topical apraclonidine and brimonidine was not dependent on intact sympathetic innervation.

– Activation of postsynaptic α_2-receptors in the ciliary epithelium. The presence of these receptors has been shown in the non-pigmented ciliary epithelium (NPE) and the ciliary muscle (Huang et al. 1995). It is hypothesized that through the stimulation of α_2-receptors on NPE, α_2-agonists inhibit the adenylate cyclase activity, which leads to depressed conversion of ATP to cAMP, which in turn would result in a reduction of AH production (Gharagozloo and Brubaker 1991). Basically, this sequence of events is highly probable because it is a well-known fact that α_2-adrenergic receptors are negatively coupled to adenylyl cyclase, which has also been revealed with respect to the ciliary body (Kintz et al. 1988). Moreover, there are reports indicating that activation of α_2-receptors in the ciliary body results in the inhibition of of cAMP production (Mittag and Tormay 1985, Crosson et al. 1992). However, as discussed previously, the question of the participation of the cAMP signalling pathway in the regulation AH production is unclear and therefore – considering the current state of knowledge – the mechanism suggested above remains in the realm of hypotheses.

– Activation of both presynaptic α_2-receptors and postsynaptic α_2-receptors in the ciliary epithelium.

Although the reduction of AH formation is the principal mechanism responsible for the α_2-agonist-induced IOP-lowering effect, there are many indications pointing to possible involvement of other mechanisms in the induction of this effect. Fluorophotometric studies have demonstrated that apraclonidine-induced reduction in IOP was not merely a result of the depressed AH production, but was also associated with an increase in the fluorophotometric outflow facility and a decrease in the episcleral venous pressure (Toris et al. 1995b). In the light of this finding, apraclonidine reduces IOP through multiple mechanisms.

Based on the cited research results, it is claimed (Toris 2010) that one of the mechanisms responsible for the IOP-lowering effect of apraclonidine is an increase in the trabecular outflow facility. This, however, has not been confirmed by other researchers, and furthermore, in the quoted research it was not proven that apraclonidine had a meaningful effect on the tonographic outflow facility (Toris et al. 1995b). In addition, other scientists did not demonstrate the effect of apraclonidine and brimonidine on the AH outflow resistance (Maus et al. 1999). Some authors undermine the influence of apraclonidine on the trabecular outflow, while emphasizing that the drug enhances the uveoscleral outflow (Bartlett et al. 2008). It is generally accepted (Bartlett et al. 2008, Stamper et al. 2009) that one of the mechanisms of action underlying the ocular hypotensive effect of brimonidine is the increased outflow of AH through the uveoscleral pathway, which is verified by results of investigations on both human subjects (Toris et al. 1995a, 1999) and animal models (Burke and Schwartz 1996). One study suggests that the brimonidine-induced reduction in IOP is associated initially with a decrease in AH production, and after chronic treatment with an increase in uveoscleral outflow (Toris et al. 1999). Toris (2010) points to two hypothetical mechanisms, which might be responsible for the brimonidine-induced enhancement of uveoscleral outflow:

– Brimonidine enlarges the interstitial spaces within the ciliary muscle. This hypothesis is supported by results indicating that α_2-agonists relax the ciliary muscle (Kubo and Suzuki 1992).

– Brimonidine improves production and release of endogenous prostaglandins which in turn may reduce the volume of the extracellular matrix filling spaces between the ciliary muscle bundles. This hypothesis relies on the fact that adrenergic agonists stimulate the release of prostaglandins into the anterior chamber *in vivo* and the synthesis of prostaglandins in ocular tissues *in vitro* (Camras and Podos 1989).

The above actions should result in increased fluid permeability of the ciliary muscle interstitial spaces, and thus in reduction of the hydraulic resistance in the uveoscleral outflow.

Experimental studies have demonstrated that brimonidine exerts neuroprotective action in animal models of optic nerve injury relevant to glaucoma including partial optic nerve crush, chronic ocular hypertension and retinal ischemia (Yoles et al. 1999, Donello et al. 2001, WoldeMussie et al. 2001, Mayor-Torroglosa et al. 2005). However clinical trials have failed to translate into similar efficacy in humans (reviewed in Saylor et al. 2009).

Effect on IOP

The available literature comprises just three reports dealing with the effect of topical application of α_2-agonists on IOP in small animals (Miller et al. 1996, Miller and Rhaesa 1996, Gelatt and MacKay 2002). Single topical administration of apraclonidine (0.5%) in normotensive canine eyes lowered mean IOP by 3.0 mm Hg (16%) 8 h after treatment (Miller et al. 1996). Apraclonidine also significantly decreased IOP in clinically normal cats (Miller and Rhaesa 1996). The drug lowered IOP a mean of 4.8 mm of Hg (24.0%) 6 h after treatment. Unfortunately, administration of apraclonidine to cats was associated with a reduction in the heart rate and other undesirable systemic side effects, which is why the cited authors claim that apraclonidine is too toxic to be given to this species (Miller and Rhaesa 1996). In a study on the effect of single and multiple doses of 0.2% brimonidine in glaucomatous Beagles, the drug was not determined to depress IOP statistically significantly, although it demonstrated some tendency towards that direction (Gelatt and MacKay 2002).

Clinical use

In people, 0.5% apraclonidine is typically used for short-term adjunctive therapy in patients not controlled with other ocular hipotensive agents, whereas 1% concentration is indicated for the prevention of postoperative IOP elevation following anterior segment laser surgery (Marquis and Whitson 2005). The long-term use of apraclonidine is limited due to the high incidence rate of ocular side effects, including allergic conjunctivitis (Butler et al. 1995). For chronic use of α_2-agonists to lower IOP, brimonidine has become the much more commonly used agent (Reynolds 2009); it is used as mono- or, more typically, adjunctive therapy (Marquis and Whitson 2005). It is also effective for prevention of postoperative IOP elevations following anterior segment laser surgery (Marquis and Whitson 2005). However, this should not be translated to medical treatment of glaucoma in small animals. In view of the previously cited results of Miller and Rhaesa (1996), the side effects in general preclude the use of apraclonidine in cats (McLellan and Miller 2011). Considering the results of a retrospective study reported by the ASPCA Animal Poison Control Center (Welch and Richardson 2002), the administration safety of 0.2% brimonidine in dogs also raises doubts. Taking into account the relatively low efficacy of apraclonidine (Miller et al. 1996) and absence of any evident effects produced by brimonidine (Gelatt and MacKay 2002) on lowering IOP in dogs, it

becomes clear that these drugs do not play any significant role in the therapy of glaucoma in small animals. This is further confirmed by the fact that α_2-agonists are not recommended for the treatment of glaucoma in veterinary patients (Gelatt et al. 2007, Miller 2008, Martin 2010). These drugs are not included in the commonly approved clinical algorithm for the management of different types of glaucoma in dogs and cats (Miller 2008). Because of some albeit low efficacy of apraclonidine (0.5%) in lowering dogs' IOP, Miller et al. (1996) have suggested that it can be a useful adjunct to other antiglaucoma treatment modalities in dogs, which at present seems to be the only clinically justifiable use of this drug in dogs. These authors have pointed out that apraclonidine (0.5%) is unlikely to be effective as the sole agent in most forms of canine glaucoma (Miller et al. 1996).

Side effects

Topical application of apraclonidine caused ocular or extra-ocular side effects both in dogs (Miller et al. 1996) and cats (Miller and Rhaesa 1996). In dogs, the most evident adverse ocular effect was mild blanching of the conjunctiva and mydriasis (Miller et al. 1996). The most likely cause is the stimulation of α_1-adrenergic receptors in the conjunctival arterioles and the iris dilator muscle, respectively. In people, the use of apraclonidine is also associated with possible occurrence of mydriasis (Barlett et al. 2008), although in cats the medication can produce a reverse effect. Miosis occurred in 46% of the apraclonidine-treated feline eyes (Miller and Rhaesa 1996). Furthermore, local side effects in cats included mild blanching of the conjunctiva and blepharospasm (Miller and Rhaesa 1996).

The topical application of brimonidine and apraclonidine may decrease heart rate in small animals. It has been demonstrated that brimonidine (0.2%) produced a reduction in heart rate (12-22%) in dogs (Gelatt and MacKay 2002). Instillation of apraclonidine (0.5%) reduced resting heart rate (9 to 19.5%) in individual dogs in one study; however, the mean heart rate was not significantly reduced when all dogs were viewed as a group (Miller et al. 1996). It seems that cats are more sensitive to apraclonidine-induced bradycardia, because administration of this drug to feline patients involved the appearance of a mean reduction in heart rate by about 12% (Miller and Rhaesa 1996). Moreover, most of the treated cats (8/9) vomited after instillation of the drug, and some continued to vomit for up to several hours post-treatment. This effect is probably mediated by stimulating α_2-receptors placed in the chemoreceptor trigger zone of the area postrema (Hikasa et al. 1992). A retrospective study was conducted of brimonidine ophthalmic solution ingestion in 52 dogs reported to the ASPCA Animal Poison Control Center. Incidence of clinical signs were bradycardia (67%), depression (46%), ataxia (27%), hypotension (25%), pallor (23%), weakness (17%), change in mucous membrane color (17%), hypothermia (13%), vomiting or retching (13%). Shock, weak pulses, and poor capillary refill time were also reported (Welch and Richardson 2002). It has been concluded that due to the possibility of severe cardiovascular effects developing, the ingestion of brimonidine ophthalmic solution in dogs should be considered dangerous (Welch and Richardson 2002).

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